Phytochemical Investigation of the Medicinal Plant *Acacia ferruginea*- Search for Anti-Quorum Sensing Agents Against *Pseudomonas aeruginosa*

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Medicinal plants are important sources and have been used for thousands of years for curing health conditions, flavoring, preserving food, and preventing diseases, including epidemics. Active compounds engendered during secondary metabolism are typically responsible for the biological properties of plant species (Singh *et al*.,2015). In plants, approximately 50,000 metabolites have been discovered, with the total number expected to reach 200,000 (Sivanadham *et al*.,2015). Modern research has grasped its success and has employed numerous natural remedies known to ancient civilizations and utilized in modern pharmacotherapy for millennia. (Petrovska *et al.*,2012). Traditional medication, primarily derived from plants, has played a critical role in keeping humans disease-free on our planet. The World Health Organization (WHO) reported that 80% of the world's population use herbal medicines for their primary healthcare. Natural products play a vital role in treating and preventing infectious diseases and many approved drugs that we are using nowadays are derived from medicinal plants. Till date, several studies have been focused on herbs that play a major role in prevention, management and treatment of various diseases due to their efficacy, less side effects, and relatively cost effectiveness (Pandey *et al*.,2014). As medicinal plants are important medical resource in maintaining the human health, through standardizing and testing the safety of active plant-derived compounds will lead to the advent of a new age in health and benefit people.

According to WHO, resistant microorganisms such as bacteria, fungi, viruses, and parasites can combat antimicrobial drugs, which leads to ineffective treatment resulting in persistence of infections. Although the development of multi drug resistance is a natural phenomenon, its huge rise in recent years has become a major threat to the mankind especially among in the immunocompromised individuals. The process of drug discovery of new antimicrobial drugs takes a long time. Hence only very few new agents have recently been approved by FDA and are available for use. The Infectious Diseases Society of America (IDSA) recognizes antimicrobial resistance as "one of the greatest threats to human health worldwide". Hence, the identification and evaluation of new antimicrobials with alternate strategy is much warranted.

Quorum sensing (QS) is a cell-to-cell communication process in bacteria to stimulate and respond based on population density through small signalling molecules (Jomehpour *et al*.,2013). Bacteria use QS systems to coordinate certain behaviors such as biofilm formation, virulence and antibiotic production. Recently, quorum sensing has been shown to be involved in the development of resistance to various antimicrobial treatments and immune modulation. A drug which is capable of blocking QS is likely to increase the susceptibility of the infecting organism to host defenses and its clearance from the host. Since pathogenicity in many bacteria is regulated by QS, inhibition of this mechanism may lead to the suppression of virulence (Bacha *et al*.,2016). Quorum sensing inhibitors do not kill bacteria like conventional antibiotics, there is less chance of resistance developing due to a lack of selective pressure. As a result, they appear

to be appealing, especially for multi-antibiotic resistant bacterial pathogens, and they could be developed as a broad spectrum anti-infective in the future.

The attenuation of bacterial pathogenicity by quorum sensing inhibitors is highly significant particularly in respect to the emergence of the multi-antibiotic resistant bacteria *P. aeruginosa.* Interference with quorum sensing could be a novel approach to control bacterial infections as many bacteria rely on quorum sensing (Jiang *et al.*,2019). Many medicinal plants and their secondary metabolites have been identified as promising quorum sensing inhibitors and were proven based on studies. Pyocyanin was remarkably reduced by *Aegle marmelos* and enhanced cell adhesion inhibition was shown by *Cynodon dactylon* (Gala *et al*.,2013). Plants from 61 distinct families were assessed for quorum sensing especially plants belonging to the families Fabaceae, Lamiaceae, Combretaceae, Myrtaceae, Zingiberaceae are widely studied and they potentially inhibit quorum sensing.

The rationale behind this study is the need to alleviate the effect of multi drug resistance by adopting alternate strategy of quorum sensing using medicinal plants and their constituent molecules as potential quorum sensing inhibitors.

The plant *Acacia ferruginea* grows to a small tree and belongs to the Mimosaceae family, a unique natural source widely used in the traditional systems of medicine to treat various ailments. In Tamil it is called as vanni, karambai and velvelam. Mostly this plant species grows in the southern and eastern parts of India. Decoction of leaves of this plant is used internally to cure mouth ulcers, boils and as topical application to cure wounds and skin infections. The present investigation is beneficial to conserve *A. ferruginea* as it comes under endangered plant species due to overexploitation and environmental conditions. Since there has been no significant scientific evidence on the pharmacological potential and medicinal uses of this genus, the current study is conducted to resolve this lacuna by phytochemical and physicochemical (qualitative and quantitative) assessments.

Aim and Objectives

The primary focus of this study is to find quorum sensing inhibitors against *P. aeruginosa* from a chosen medicinal plant and the key objectives are

- To identify anti-quorum sensing (AQS) active fractions from the medicinal plant *Acacia ferruginea*
- To isolate and characterize the chemical constituents from the most active fractions
- To validate the anti-quorum sensing inhibition effect of the constituents
- To evaluate the efficacy of the identified quorum sensing inhibitors on quorum sensing regulated genes
- To validate the anti-quorum sensing ability of select molecules against quorum sensing targets

Medicinal Plant Chosen for the Study

Aca*cia* species are multipurpose trees distributed worldwide comprise nearly 1200 species of the *Fabaceae* family belonging to the tribe *Acaciacea.* About 152 chemical components have been identified from the *Acacia* species during the last seven decades. One of the species, *Acacia ferruginea*, belongs to *the Mimosaceae* family, a unique natural source widely used in the

traditional systems of medicine to treat various ailments. It has been reported that it is commonly used in folk medicine for the management of inflammation, pain, cancer cure and to treat numerous ailments including hemorrhage, leprosy and irritable bowel syndrome *via* various pharmacological actions (Sowndhararajan *et al.*,2015, Sakthivel *et al.,* 2016, Bukhari *et al.,* 2010). There were some protective effects in using *A.ferruginea* extract against cyclophosphamide induced toxicities ,due to higher anti-oxidant capability (Sakthivel *et al.*,2014). The work aimed to provide the anti- virulent properties of *A. ferruginea* DC., and to evaluate the active constituents by quorum sensing inhibition studies. Since many of the compounds reported in *Acacia* species were matched with the already reported quorum sensing inhibitors we hypothesized that being an *Acacia* genome *A. ferruginea* may contains compounds with therapeutic property which can acts as quorum sensing inhibitors. The stem-bark and leaves part were taken for the study.

The research study will be presented in five chapters as follows.

Chapter I: A brief introduction to the study and a mention of its prime focus

Chapter II: Review of Literature

Review of literature pertaining to the study was done in two major aspects

Review on natural quorum sensing inhibitors against *P. aeruginosa*

Extensive literature was collected from scientific databases such as Scifinder, pubmed, google scholar. The anti-quorum sensing activity of medicinal plants against *P. aeruginosa* was reviewed for the period 1997-2019 which involved the comparison of AQS activity of various extracts of plants against *P. aeruginosa*, plant parts highly studied for AQS activity, extraction methods adopted, and the AQS efficacy of the isolated phytochemicals.

A thorough literature review on the medicinal plant *Acacia ferruginea*

Literature was reviewed for the period 1970-2019. Despite the plant's folkloric use and tremendous therapeutic potential, only twenty two research findings were found published. All reports on the plant have been reviewed and will be presented in the following section in the thesis

- Qualitative phytochemical analysis on the plant extracts
- Quantification of phytochemical content
- Chemical constituents
- Biological activities

Chapter III*:* Methodology

Methodology of research work will be presented in five phases

Phase I: Phytochemical Profiling of the Medicinal Plant *Acacia ferruginea*

Phase II: Chemical Investigation of Extracts-Isolation of Chemical Constituents

Phase III: Synthesis of Silver Nano Particles Using *A. ferruginea* Extracts and their Characterization

Phase IV: Biological Studies

Phase V: Computational Studies

Authentication of Plant

The leaves and stem-bark of *Acacia ferruginea* were collected from the village Nalligoundanpalayam (11012′19.2″ N 77017′55.3″ E), Avinashi, Tamil Nadu state, India. The plant was authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu, India (BSI/SRC/5/23/2018/ Tech/2080). The pre-cleaned leaves and stem-bark were dried under shade and pulverized for extraction.

Extraction of Plant Material

Plant material (0.20 Kg) was shade dried, pulverized and sequentially extracted with solvents of increasing polarity (Hexane, ethyl acetate, ethanol, 90% ethanol, and distilled water) by soxhlation method. The extracts were concentrated by distillation or rotary evaporation as the case may be. While aqueous extracts were concentrated by lyophilisation. The extract concentrates were designated as below.

Designation of Extracts	
Hexane extract of stem stem-bark of Acacia ferruginea	**AFBH**
Hexane extract of leaves of Acacia ferruginea	**AFLH**
Ethyl acetate extract of stem stem-bark of Acacia ferruginea	**AFBEA**
Ethyl acetate extract of leaves of Acacia ferruginea	**AFLEA**
Ethanol extract of stem stem-bark of Acacia ferruginea	**AFBE**
Ethanol extract of leaves of Acacia ferruginea	**AFLE**
Hydroethanol extract of stem stem-bark of Acacia ferruginea	**AFBHE**
Hydroethanol extract of leaves of Acacia ferruginea	**AFLHE**
Aqueous extract of stem stem-bark of Acacia ferruginea	**AFBAq**
Aqueous extract of leaves of Acacia ferruginea	**AFLAq**

Phase I: Phytochemical Profiling of the Medicinal Plant *A. ferruginea*

Phytochemical profiling of the plant comprise the following stages of the work and standard methods were adopted for the study

- **Physico-chemical characterization** (extractive values, organoleptic properties, solubility and fluorescence characteristics)
- **Proximate analysis** (ash value, extractive value, moisture content, volatile matter and metal content analysis)
- **Determination of nutritional parameters** (crude fiber, fat, protein content)
- **Phytochemical characterization** (qualitative and quantitative)
- **Chemical fingerprinting of extracts** (UV-Vis, FT-IR, NMR)
- **Chromatographic fingerprinting of extracts** (TLC, HPTLC, GC-MS, HR-LCMS)

Standard methods of analysis have been adopted as per Ayurvedic Pharmacopeia and WHO guidelines for herbal drugs with relevant modifications wherever necessary (API 2016; WHO 1992)

Phase II: Chemical Investigation of Extracts-Isolation of Chemical Constituents

Isolation of constituents of stem stem-bark and leaves of the chosen plant *A. ferruginea* was done as outlined in the charts 1 and 2. The hexane extract of the leaves of the plant was subjected to preparative TLC and cyclograph-aided radial separation. The hydroethanolic extract of the leaves was subjected to column chromatography and the hexane extract of the Stem stem-bark was analysed by PTLC for separation of constituents.

- **Column Chromatographic Analysis of Hydroethanol Extract of Leaves**

The hydroethanol extract of *Acacia ferruginea* leaves (25g) was dissolved in minimum volume of methanol and made into slurry with minimum amount of silica gel. The slurry was subjected to chromatographic separation over a column of silica gel (100 g) built in hexane and eluted with (i) hexane (ii) hexane and chloroform mixtures with increasing amounts of chloroform, (iii) chloroform and methanol mixtures with increasing amounts of methanol. Eluates of 200 mL were collected and the solvent was recovered by distillation. The homogeneity of the fractions was examined by TLC. The spots developed were visualized under UV light and then by exposure to iodine vapor. Similar fractions were combined. The outline of the column chromatographic separation is presented in chart 1.

- **Acetone Treatment**

The hexane extracts of both *A. ferruginea* leaves and stem stem-bark were separately treated with acetone to obtain an insoluble compound. The hexane extract of the leaves was treated with acetone and the insoluble compound was separated and designated as AFI.

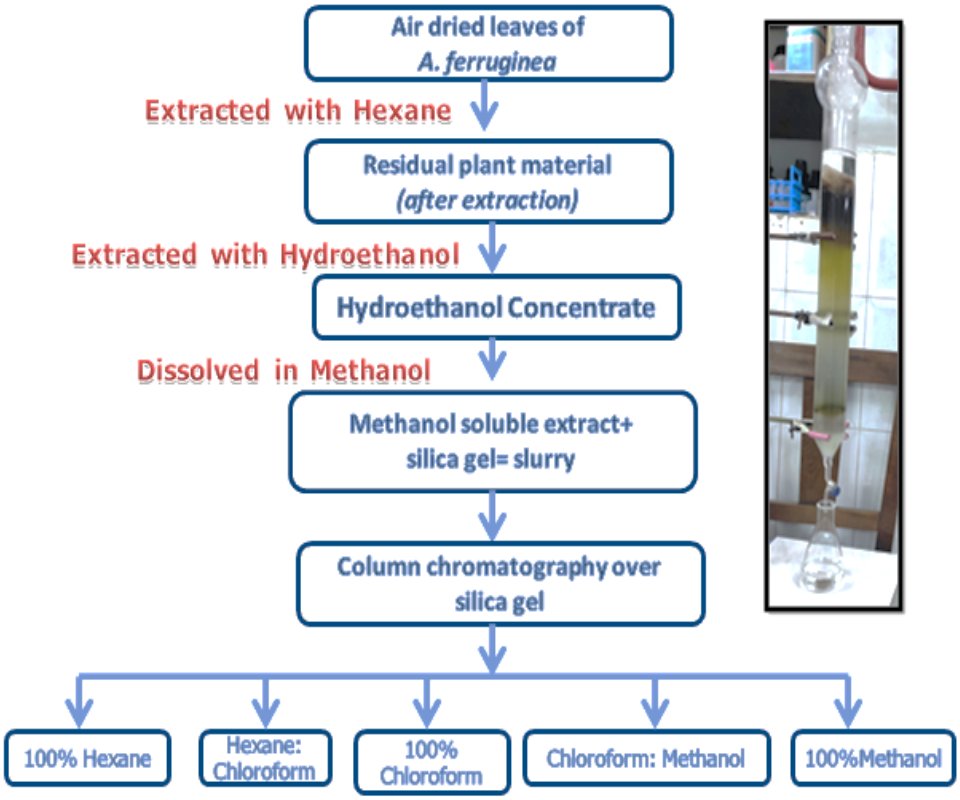

Chart 1: Outline of Procedure for Isolating Chemical Components from *A. ferruginea* Leaves by Column Chromatography

- **Preparative Thin Layer Chromatography of Hexane Extracts (PTLC) of Leaves and Stem Stem-bark**

Hexane extracts of leaves and stem stem-bark were selected for PTLC study. After acetone treatment the acetone soluble fraction of leaves was subjected to PTLC. The optimized solvent system hexane and ethyl acetate was used to separate the compounds. About one gram of each sample is treated with 10 mL of acetone and the soluble portions were collected to proceed with preparative TLC and the insoluble portions were washed and kept separately for further analysis. The mixture was then centrifuged to separate from silica and the obtained supernatant was removed by syringe filtration technique and stored.

- **Cyclograph -Aided Radial Separation of Leaf Hexane Extract**

The acetone soluble portion of the leaves hexane extract is subjected to Cyclograph aided radial separation. To perform this cyclograph aided radial separation process, a centrifugally accelerated device “Cyclograph” is used. About 1 g of AFLH extract was mixed with 10 mL of the optimized solvent system. The extract was well dispersed and filtered using micro syringe filter and applied to the Silica Gel GF 2mm Pre-Scraped Rotor by passing through the solvent pump with flow rate 2-4 mL/min to achieve high resolution. The rotor is initially treated with

hexane and then with optimized mobile phase prior to sample application. The elution is carried out using optimized solvent system. The outline of the procedure is given in the chart 1.

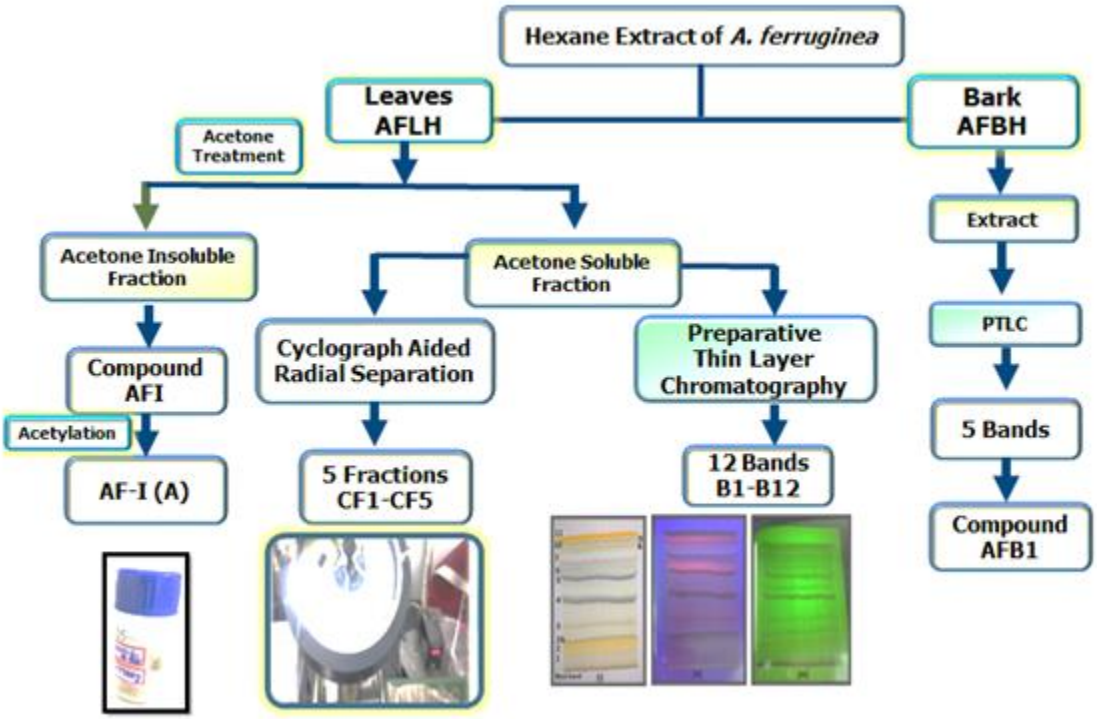

Chart 2: Outline of Procedure for Isolating Chemical Components from *A. ferruginea* Leaves and Stem Stem-bark Using Preparative TLC and Radial Separation

- **Characterization of Chemical Constituents**

The isolated compounds were characterized by preliminary tests, physical data and spectral analysis. Spectral techniques used for structure elucidation include UV,FT-IR, NMR (1D and 2D) and mass spectrometry.

Phase III: Synthesis of Silver Nano Particles Using *A. ferruginea* Extracts and their Characterization

Plant-mediated synthesis of silver nanoparticles (AgNPs) has emerged as a feasible alternative to traditional chemical synthesis and its popularity is growing due to its eco-friendly, easy fabrication, and cost-effective features. In this study, the aqueous (Aq) and ethanol (E) extracts of leaves (L) and stem stem-bark (B) extracts of *A. ferruginea* (AF) were used to synthesize silver nanoparticles (AgNPs) under sunlight. The respective AgNPs are coded as

Designation of synthesized silver nanoparticles	
AFLENPs.	Silver nanoparticles from leaf ethanol extracts
AFLAqNPs	Silver nanoparticles from leaf aqueous extracts
AFBENPs.	Silver nanoparticles from stem-bark ethanol extracts
AFBAqNPs	Silver nanoparticles from stem-bark aqueous extracts

The synthesized silver nanoparticles were characterized using various spectral techniques (UV–vis, FTIR, XRD, FE-SEM, EDS, HR-TEM, DLS and zeta potential).

Phase IV: Biological Studies

Antioxidant and anti quorum sensing assays for the sequential extracts, main fractions, isolated compounds and biosynthesized nano particles were done. The total antioxidant activity (TAA) and DPPH radical scavenging activity were adopted to assess the antioxidant activity. Anti quorum sensing was done by disc assay, biofilm inhibition, growth curve analysis, and violacein inhibition assay. The following bacterial strains were purchased from MTCC (The Microbial Type Culture Collection and Gene Bank) and used for the studies.

1. *Chromobacterium violaceum* MTCC 2656
2. *Pseudomonas aeruginosa* PAO1 – MTCC 3541

Appropriate culture medium will be used to grow and study various parameters.

Phase V: Computational Studies

The *insilico* studies validates the quorum sensing inhibition potential of compounds predicted by GC-MS and LC-MS of ethanol extracts of *A. ferruginea* plant. Further studies will be done for compounds. The structure of compounds was docked with the 2D structures of differentially expressed protein products of the genes and the interaction strengths were accomplished using maestro v.11.8 from Schrodinger 2018 Drug Designing Software.

Chapter IV: Results and Discussion

The results pertaining to the present work will be presented in the Chapter IV.

Phase I: Phytochemical Profiling of *Acacia ferruginea*

Phytochemical profiling will be of significance in preparing a monograph on this medicinal plant. The salient results are presented below.

- **Physicochemical Characterization**

The hexane extract of both stem-bark and leaves is completely soluble in hexane and chloroform, the polar extracts are highly soluble in ethanol and green and greenish brown colour were observed under UV when the stem-bark powder was treated with picric acid and ferric chloride. These characteristics aid in identification of the authentic plant material and to test for any adulterating material methanol. The high dry content value and low moisture content of the leaves indicate lower decay rate and resist fungal attack and that the leaves are more suitable for herbal formulations. Metal content analysis indicates the abundance of organic compounds in the plant and there are no toxic metals.

- **Phytochemical Characterization**

The presence of alkaloids, phenolics and terpenoids was indicated by colour tests. A notable fact is the high volatile content of both stem-bark and leaves of the plant. This probably indicates that the plant may be a source of essential oils and other low volatile metabolites.

- **Spectral Fingerprinting of Extracts**

The UV-VIS spectrum of the hexane extract of stem-bark indicated only saturated metabolites but the hexane extract of leaves indicated an additional band at 410 nm due to unsaturated moieties. The polar extracts indicated presence of flavonoid and other polar metabolites. Peaks with excitation wavelength 400-490 nm were observed for the aqueous extracts of leaves. Based on the fluorescence studies it was noted that both the leaf and stem-bark extracts are rich in fluorophores. The UV, IR and NMR spectral fingerprints were recorded and the spectra will be presented in the thesis.

- **Chromatographic Fingerprinting of Extracts**

TLC Analysis: TLC analysis of the extracts was done with various solvent systems using hexane-ethyl acetate mixtures and optimized.

HPTLC: HPTLC analysis of extracts of *A. ferruginea* leaves and stem-bark was carried out as a fingerprinting for documentation. Both stem-bark and leaf ethanolic extracts show a prominent peak with Rf of 0.75 and compared with that of standard flavonoid quercetin.

GC-MS Analysis: From the GCMS analysis of the extracts, the probable compounds were identified based on the NIST database. Six probable compounds were identified for each of the AF leaf extracts.

HR-LCMS: HR-LCMS analysis was carried out for the hexane and ethanolic extracts of leaves and stem-bark of *A. ferruginea* and will be presented in the thesis. The number of major peaks indicated the number of major constituents present in each extract the analysis of the major constituents was taken up by database comparison.

The leaves and stem-bark of *Acacia ferruginea* are rich in pharmaceutically important phytochemicals and may be prospective agents for curing oxidative stress and quorum sensing related infections.

Phase II: Chemical Investigation of Extracts-Isolation of Active Constituents

- **Column Chromatographic Isolation of Hydroethanol Extract of Leaves**

Column chromatographic isolation of hydroethanol extract of leaves of *A. ferruginea* led to the isolation of 11 compounds. Totally 461 fractions were collected and based on TLC similar fractions were combined and thirty main fractions were obtained. Work up of the main fraction led to isolation of eleven compounds designated as C1,C2,C3,C4,C5,C6,C7,C8,C9,C10 and C11. Of the 11 compounds isolated form the leaf ethanol extract, except C1, all the other compounds were completely soluble in polar solvents, methanol, DMSO and water. The compounds C2 to C8 were identified as flavonoid class of compounds. Characterization of the major compounds was done by spectral analysis. It was observed by UV and spectral analysis with shift reagents that all the indicated major compounds gave a significant bathochromic shift in sodium methoxide and with ferric chloride indicating presence of phenolic hydroxyls and chelated hydroxyl respectively. A shift in sodium acetate indicates presence of 7- hydroxylation. There

was no bathochromic shift with boric acid indicating absence of dihydroxylated system in the flavone moiety. This shift reagent analysis aided in suitably assigning the functionalities in the compounds. The complete characterization of compounds will be presented in the thesis.

- **Preparative Thin Layer Chromatography of Hexane Extracts**

The acetone soluble fraction of leaves and stem-bark was subjected to PTLC. Preparative thin layer chromatography of leaf hexane extracts led to the isolation of 12 bands from and 5 bands from stem-bark hexane extract and major bands were taken for further studies. The bands were analysed for quantity, appearance, colour, TLC, UV and fluorescence nature for both stem-bark and leaves. The UV spectral overlay indicated that except one band (B)8, all other bands had similar UV-visible absorption corresponding to lutein class of compounds. Each band corresponds to a single compound and the characterization details based on spectral analysis will be presented in the thesis. The PTLC fraction of the stem-bark hexane extract yielded a pale white solid designated as AFB1. Spectral analysis and literature data led to identification of AFB1 as lupeol. The presence of lupeol as a major compound of the stem-bark of this plant bestows anti-inflammatory potential. The stem-bark extract may be used in preparing wound healing ointments also.

- **Cyclograph -Aided Radial Separation of Leaf Hexane Extract**

Cyclograph -aided radial separation of hexane extract of leaves of *A. ferruginea* led to the separation of 15 fractions totally and based on TLC results similar fractions were combined to give 5 main fractions designated as CF1,CF2,CF3,CF4,-CF5. The UV spectra of the cyclograph fractions indicates a similar chromophoric system in fractions CF2,CF3,CF4 and CF5. The peaks around 2800 - 2900cm^{-1} indicate that the metabolites are saturated molecules mostly. The fractions were further subjected to GCMS analysis to identify probable compounds. The TLC of these fractions was also compared with that of the PTLC fractions of the leaf hexane extract to establish similarity and the discussion will be presented in the thesis.

Phase III: Synthesis of Silver Nano Particles Using *A. ferruginea* Extracts and their Characterization

Silver nanoparticles were synthesized from the ethanol and aqueous extracts of the leaves and stem-bark of *A. ferruginea.* Stem -bark extracts produced violet AgNPs and leaf extracts produced wine red colour AgNPs. The UV–Vis spectra of the synthesized AgNPs shows an absorption peak in the range 420 nm to 457 nm characteristic of the formation of the silver nanoparticles. The fluorescence analysis shows the emission spectrum of 779 and 894 nm at 390 and 450 nm excitation wavelengths for AFBAqNPs and AFBENPs, respectively. From HR-TEM images the size of AFBAqNPs, and AFBENPs was noted as ~ 19.73 and 12.33 nm respectively. The AFBENPs have a bimodal size and distribution, with smaller spherical particles of size 2.60 nm. Zeta potential of AFBAqNPs and AFBENPs is − 26.1 mV and 5.9 mv respectively indicating the stability of nanoparticles. The salient results are presented in the table below

Sample	Zeta potential (mV)	PSA Z- average (nm)	FE-SEM (nm)	HR-TEM (nm)
AFLENPs.	22.3	180.5	37.72	19.35
AFLAqNPs	-9.4	54.1	79.83	13.66
AFBENPs.	-26.1	55.0	35.8	12.33
AFBAqNPs	81.9	110.5	162.2	19.73

All the synthesized AgNPs have crystalline nature and face-centered cubic (FCC) structure. From the fluorescence analysis the excitation wavelength dependency was assessed. The fluorescence intensified in the nanoparticle host solution indicating the presence of fluorescent molecules.

Phase IV: Biological Studies

Phase IV comprises of biological activity studies with focus on assessing the anti-quorum sensing efficacy of the sequential extracts, column main fractions, PTLC fractions, cyclograph fractions and all the isolated compounds. Total antioxidant activity **(**TAA) of the various extracts of *A. ferruginea* leaves and stem-bark was measured with ascorbic **a**cid as standard. The extracts from the stem-bark were found to exhibit higher anti-oxidant potential compared to leaves except the leaf ethanol extract. This indicates a higher content of anti-oxidant flavonoid molecules in the leaves probably.

The percent DPPH scavenging activity of the leaf extracts ranged from 5% to 74% and stem-bark from 14% to 78% at the concentration range of 25-200 µg/mL. A significant correlation exists between total antioxidant activity and DPPH scavenging activity, and the results are comparable. The strong DPPH scavenging activity of *A. ferruginea* stem-bark suggests that the leaves contain more phenolics that may be attributed to the antioxidant properties of *Acacia* species .The stem-bark nanoparticles of *A. ferruginea* show 56% and 64% for ethanolic and aqueous extracts respectively and both has dose dependent effect. Disc diffusion assay was carried out for all the sequential extracts and significant results were noted for ethanolic extracts of both stem-bark and leaves. The ethanol extract of *A. ferruginea* leaves and stem-bark significantly reduced the biofilm formation by about 69.81% and 67.84% respectively. At 200 and 400 µl the biofilm inhibition of stem-bark extract was found to be higher, whereas the inhibition was found to be less for all other concentrations when compared to leaf extract. The ethanolic extract of leaves and stem-bark has potentially suppressed the biofilm formation as well as the violacein pigment in a concentration dependent manner. As there is no significant growth inhibition was found, both the AFBE and AFLE of *A. ferruginea* express aptness to serve as quorum sensing inhibitors rather than antibiotics.

It is evident that *A. ferruginea* has versatile chemical profile and the results showed that the *A. ferruginea* has potential anti-quorum sensing activity. *A. ferruginea* leaf hexane extract PTLC fractions bands 3, 10,11, CTLC fraction 1,a major band from stem-bark hexane extract and AFBNPs exhibited significant reduction in QS-mediated violacein pigment production in disc assay. Zone of inhibition exhibited by chromatographic main fractions of leaf ethanol extract

F22, F25 and F30 is comparable with that of standard. For the synthesized nanoparticle containing host solution significant activity was observed against *P. aeruginosa* strains at higher concentrations and moderate activity against *C. violaceum* strains. The study indicates that the extracts and the isolated compounds possess higher quorum sensing inhibiting potential compared to the nanoparticle containing host solution.

The quorum sensing inhibition of the compounds isolated from the leaf ethanol extract against *C. violaceum* will be reported in the thesis.

Phase V: Computational Studies

Docking analyses of 19 compounds as predicted from GC-MS analysis against CviR, a quorum sensing regulatory protein was performed and higher binding affinity was found in severin when compared to the native ligand C6-HSL. Based on molecular docking studies 6 hits were found to have potential interaction with CviR protein. Severin was able to form hydrogen bonds with binding sites with highest G score and interacted better with the quorum sensing system of *C. violaceum*.

All data on phytochemical profiling of leaves and stem-bark of *A. ferruginea* will be documented as a ready reckoner document sheet similar to the Ayurveda plant monograph and will be presented in the thesis.

Chapter V: Conclusion

In the present research study a medicinally important plant Acacia ferruginea was taken up for phyto chemical investigation with focus on finding anti quorum sensing inhibitors. The study led to isolation of **eleven** compounds from the hydroethanolic extract of leaves - Identified as flavonoid glycosides and phenolics and characterised. **Four** major compounds isolated from the leaf hexane extract have similar structural systems of the lutein pigments. One major compound from the stem-bark hexane extract identified as lupeol. The extracts can be proposed to be the source of quorum sensing inhibition agents. The compound AFB1 isolated from the stem bark hexane extract, the compound CFC1 isolated by radial separation, the bands B3, B8 and B11 separated by PTLC of the leaf hexane extract are found to express quorum sensing inhibition. The column main fractions MF 24, 25, Compound C11 show efficacy comparable to standard. Extracts are also found to be source of fluorophores as evidenced by fluorescence potential. *A. ferruginea* mediated nanoparticles revealed that AgNPs of suitable extracts and also effective QS agents.

The research study validates the chosen plant *A. ferruginea* as a potential source of anti-quorum sensing inhibitors.

Paper Published

- Jeevitha M and Shubashini K. Sripathi*, Medicinal Plants and Phytochemicals Against *Pseudomonas aeruginosa* Quorum Sensing , *IJPSR*, 2020; Vol. 11(10): 4749-4773.
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Paper Accepted

- Potential Quorum Sensing Inhibitory Activity of *Acacia ferruginea* against *Chromobacterium violaceum*

Paper Communicated

- GC-MS profiling and anti-quorum sensing screening of leaf and bark extracts and fractions of *Acacia ferruginea*

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